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THE CATALASE OF SOILS

BY

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THE CATALASE OF SOILS.

It has been known for many years that certain vegetable and animal tissues contain an enzym that has the faculty of evolving oxygen from hydrogen peroxide. When in 1899 one of us studied the presence of catalase in various vegetable objects in conjunction with O. Loew, (1) then physiological chemist in the United States Department of Agriculture, it was observed among other things, that mold fungi and bacteria abound in that particular enzym. Since these lower organisms occur widely spread in soils it was not surprising to observe there also the presence of catalase. Several samples of soils treated at that time with neutralized hydrogen peroxide by O. Loew gave off free oxygen, as was to be expected.

The results reported indicate the wide extension of catalase throughout the organic world. In all materials tested of an organic nature it was found present in greater or less degree. Its persistence is indicated from the fact that museum specimens of dried leaves over 40 years of age still contained a definite amount of catalase. It seemed to be present, however, in greater amounts in those substances which were undergoing molecular changes, as in ripening fruits and fermenting substances. Soils were found to contain it in measurable amounts especially those rich in humus.

The amount of catalase found in the soil is apparently dependent upon the amount of organic matter present and the activity of the cells composing the substances. Soils where the activity of the micro-organisms is greatest are the most active in evolving oxygen when hydrogen peroxide is added. Soils that are sterile and in which organic matter is absent contain no catalase. On the other hand certain chemical elements commonly employed as fertilizers had no effect after several weeks upon the amount of catalase present. It does not seem, therefore, that the presence or absence of this enzym has any effect upon the productivity of the soil. Its absence does not interfere with the production of crops nor does it appear to be a necessity of the soil for the best production. But various conditions may enhance or depress microbial life and the result be beneficial for plant growth. (Denitrifying bacteria may cause damage in very wet soils while pathogenic bacteria as the tobacco wilt bacillus may occasionally be present.) Hence, it is of value to possess a quick and ready means to determine the relative extent of bacterial growth in soils.

A few years ago the question of the development of oxygen gas by treating soils with hydrogen peroxide was taken up by

(1) Catalase, a New Enzym of General Occurrence (Report No. 68. U. S. Dept. of Agr.)

Koenig. (2) He observed that soils in which the bacteria were killed by steam yielded much smaller amounts of oxygen than those not so treated, clearly indicating that the catalase of the soil bacteria caused the greater part of the evolution of oxygen in the soils under experiment.

Later Koenig, Hasenbaume and Coppenrath (3) investigated the power of soils for decomposing peroxide, describing it as a new property which they attributed to the enzyms existing in soils and also to the colloidal action of manganese and iron oxids. In their first work they found that different classes of soils possess this power in varying degrees, limestone soils showing the greatest activity, then clay, loam, sandy loam, and sandy soils. In later work they found that the power is in almost direct relation to the humus content of soils. It was to be expected that soils rich in organic matter or manure would yield more oxygen due to the larger amount of bacteria and mold fungi present.

In work at this Station it has been found that probably only in very exceptional cases does the colloidal effect of the mineral constituents in decomposing peroxide compare at all with the effect of the enzym. For instance, a clay subsoil of the Station containing 29.31% of ferric and aluminic oxids was almost devoid of action on peroxide. Also ignited soils gave off amounts of oxygen almost negligible compared with the quantity evolved previous to ignition. Thus it follows, that the only appreciable effect is due to enzym action. And since Loew has shown that catalase is the only enzym decomposing peroxide, this power of soils may be ascribed to the catalase content, enhanced in special cases only by colloidal action of the mineral constituents.

Since bacteria are one of the chief sources of catalase as it is found in soils, and since soils rich in humus and lime are favorable for bacterial flora it is evident why Koenig and his associates found in one instance that the catalytic power of soils was in direct relation to this humus content and in another case that limestone soils had this power to a greater extent than sandy soils.

It might be expected then, that in the amount of peroxide that soils decompose there would be a rough measure of their bacterial content. With this idea in view, a determination was made in the usual manner of the total bacterial content, both aerobic and anaerobic, of six different soils; and at the same time the relative activity of the same soils in decomposing hydrogen peroxide was determined. But it will be seen from Table I that there is no parallelism between the actual bacterial content and the activity of the soil in decomposing peroxide.

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(2) Landw. Versuch Stationen 63 page 472 (1906)
(3) " id id 69 id 401

TABLE I.

	Minutes required by 5 gms soil to evolve 100 c. c. of 0_2	Number of bacteria in 1 c. c. of soil.	Order of bacterial content by peroxide test.	Order of bacterial content by actual count.
Soil No. 1	*	3,763,000	6	4
2	13.3	11,094,000	3	2
3	18.6	4,278,000	5	3
4	9.5	94,830,000	1	1
5	17.5	1,628,000	4	5
6	12	835,000	2	6

* In 18 minutes only 9 c. c. of 0_2 evolved.

The reason for this lack of parallelism is not far to seek. In the first place, different species of bacteria contain catalase in varying amounts. Again, the enzym is much more resistant to destruction than the bacteria producing it. Soil samples kept for six months or more show the same power of decomposing peroxide as they did when first examined. Thus dead bacteria that would not appear in a bacterial count would, through the presence of their existing enzyms, have an effect in the test with peroxide. But probably the most potent factor of the disagreement lies in the presence in soils of catalase produced in vegetable cells. The decaying vegetable matter of the soil contains in itself catalase which is operative in the test with peroxide. In certain cases there might be more catalase of vegetable than of bacterial origin, so that while the bacterial count would be low, the test with peroxide would be high.

The test with peroxide, then, is a ready and rough test not only for the general bacterial content, but at the same time for the vegetable matter present in the soil. Soils rich in organic matter or bacterial life are found to show greater activity with peroxide than soils deficient in the same. While the accuracy of a chemical examination or the ordinary bacterial count is not claimed for this test, it is believed to be of value in the general examination of soils, as it can be executed with considerable ease and rapidity.

On investigating the best method of executing this test in order that uniform or comparable results might be obtained it became evident that the reaction between catalase and hydrogen peroxide was dependant upon many conditions. Furthermore, it was found that the usual methods of comparing different amounts of catalase were not at all accurate.

In some work that has been done on the decomposition of peroxide by soils, the method employed for comparison was to observe, without any particular regard to time, the amount of oxygen evolved by a certain quantity of soil from a certain amount of peroxide. This method is valueless, since, within certain limits, a small amount of catalase will decompose as much peroxide as a larger amount, although at a slower rate.

Another method has been employed in investigating the catalase content of plants and seeds. (1) In this method the quantities of catalase are compared on the basis of the number of cubic centimeters of oxygen evolved within a certain number of minutes. While this method is preferable to the former and does afford a rough comparison, it is susceptible to a large error. The inaccuracy of this method is evident from the data of the following experiment.

Three different quantities of the same soil were taken, namely, 10 gms., 7.5 gms., and 5 gms. The determinations were carried out under identical conditions of temperature, concentration, quantity of peroxide, etc. The volume of oxygen evolved was recorded every minute. The results are given in Table II.

TABLE II.

Time in minutes.	C. C. of 0_2 evolved by 10 gms. of soils.	C. C. of 0_2 evolved by 7.5 gms. of soil.	C. C. of 0_2 evolved by 5 gms. of soil.
1	28	22	17
2	48	41	31
3	66	55	43
4	80	68	53
5	94	80	63
6	105	92	72
7	117	101	80
8	125	110	87
9	132	118	96
10	139	125	101
11	145	131	108
12	152	136	114
13		142	118
14		147	124
15		150	127
16			131
17			135
18			138
19			143
20			146
21			147
22			150

Since the soil was a carefully prepared sample, the amounts of catalase present in the three cases must have been in proportion to the weights of soil, or as 10: 7.5: 5. Then if the above method is correct, the volumes of oxygen evolved in any number of minutes by the three quantities of soil should be in the ratio of 10: 7.5: 5. But taking the amount of oxygen evolved in 4, 8 and 12 minutes it can be seen that this is not so.

(1) Mass. Sta. Annual Rpt. 45 pp. 249-250
Bul. 68 U. S. D. of A.
Oesterr. Chem. Ztg. 7 (1904) N°. 8 pp 173-175.

TABLE III.

Gms. of soil.	C.C. of 0_2 evolved in 4 minutes.	C. C. of 0_2 evolved in 8 minutes.	C. C. of 0_2 evolved in 12 minutes.
10	80	125	152
7.5	68	110	136
5	53	87	114

In Table IV the comparative amounts of catalase, or, what is the same thing, the comparative amounts of soil, are calculated on the basis of the cubic centimeters of oxygen evolved in 4, 8, and 12 minutes.

TABLE IV.

Actual No. of gms. of soil.	Gms. soil estimated by vol 0_2 evolved in 4 minutes.	Gms. soil estimated by vol 0_2 evolved in 8 minutes.	Gms. soil estimated by vol 0_2 evolved in 12 minutes.
10	7.6	7.2	6.7
7.5	6.4	6.3	6.0
5	5.	5.	5.

It will be seen that the variations of the calculated from the actual quantities are so great that this method also is valueless for anything but qualitative work. A method far more accurate than either of the two preceding is to make the comparison on the basis of *time required to evolve a certain volume of oxygen from a certain quantity and concentration of peroxide.* From Table II are obtained the number of minutes required to evolve 75, 100 and 150 c.c. of oxygen, and in Table V the comparison is made on the basis of the number of minutes required to evolve these quantities of oxygen, assuming that the time required is inversely as the amount of soil or catalase.

TABLE V.

Actual No. of gms. of soil.	Gms. of soil estimated by time required to evolve 75 c. c. 0_2	Gms. of soil estimated by time required to evolve 100 c. c. 0_2	Gms. of soil estimated by time required to evolve 150 c. c. 0_2
10	9	9	9.4
7.5	7	1.1	7.3
5.	5	5	5.

Another experiment with five different weights of soil, or five different quantities of catalase, shows the accuracy that can be obtained by this method. The results are given in Table VI.

TABLE VI

Actual No. of gms. of soil	Minutes required to evolve 100 c.c. of 0_2	Gms. soil estimated by time required to envolve 100 c.c. of 0_2
10	5.3	9.9
7.5	7.4	7.1
5	10.5	5
2.5	21.1	2.5
1	49	1.1

In investigating the course of the reaction between catalase and hydrogen peroxide to find what conditions must be fixed in order to secure accuracy in the above method, it was found that the time required to evolve a certain quantity of oxygen was dependant upon:

1. The amount of catalase present.
2. The concentration of the peroxide used.
3. The amount of peroxide used.
4. The acidity or alkalinity of the solution in which the reaction took place.
5. The frequency with which the flask containing the catalase and peroxide was agitated.

In all the following experiments upon the course of the reaction the same soil was used.

(1) That the time required to evolve a certain amount of oxygen from a certain amount of peroxide is inversely proportional to the amount of catalase present, has already been shown in tables V and VI.

(2) The effect of the concentration of the peroxide can be seen from the following experiment. In this experiment 5 gms. of soil were used for each determination and 20 c. c. of the commercial peroxide, which contained 2.75% of H_2O_2. The only variation between the determinations lay in the *concentration* of the peroxide.

TABLE VII.

Solution added to 5 gms. of soil.	Concentration of H_2 0_2	Minutes actually required to evolve 150 c. c. of 0_2	On basis of No. of min. required by .92% H_2 0_2 the time calculated for other concentrations.
20 c. c. of H_2 0_2 sol, 10 c. c. Aq.	1.84%	12.8	10
ditto 20 do	1.38	14	14
ditto 40 do	.92	22	22
ditto 80 do	.55	34	33

It is apparent from Table VII that the time required by a given amount of catalase to evolve a certain volume of oxygen is inversely proportional to the concentration of the peroxide used. The minutes calculated by this law are in very close agreement with those actually obtained.

It follows as a corollary of the above, that during any single determination the amount of oxygen given off, minute by minute, is proportional to the amount of undecomposed peroxide in solution. In Table VIII are recorded the cubic centimeters of oxygen evolved during successive periods of two minutes and the concentration of the peroxide at the beginning of these periods. In the fourth column are tabulated the results obtained by dividing the figures in the second column by 23. This does not alter the ratio of the figures in column 2 but merely makes plain the parallelism between the cubic centimeters of oxygen evolved and the concentration of peroxide.

TABLE VIII.

	c. c. of 0_2 evolved during successive 2 minute periods.	Conc. of H_2 0_2 at beginning of the successive 2 minute periods.	Number of c. c. of 0_2 in second column divided by 23 for comparison
2 minutes	44	1.84	1.9
4 "	32	1.39	1.4
6 "	26	1.07	1.1
8 "	20	.81	.9
10 "	14	.61	.6
12 "	10	.47	.4

(3) Not only the concentration of the peroxide but also the amount has to be fixed in order to secure constant results with a constant quantity of catalase. For instance, 5 gms. of soil evolved 100 c. c. of oxygen from 120 c. c. of .92% peroxide in 7.3 minutes, while an equal quantity of the same soil required 10 minutes to evolve a similar amount of oxygen from 60 c. c. of .92% peroxide. The reason for this difference in time is, that, although the concentrations of the peroxide in the two determinations are equal at the start of the reaction, they do not remain so as the reaction proceeds. As oxygen is evolved the concentration of the 120 c. c. of peroxide decreases less rapidly than the concentration of the 60 c. c. Thus it is equivalent to making determinations with two different concentrations of peroxide, while the constancy of results has been shown to be dependent upon using a constant concentration of peroxide.

(4) The acidity or alkalinity of the solution in which the reaction takes place has a great influence upon the rapidity with which oxygen is evolved. The commercial peroxide has an acid reaction, which, in all the preceding experiments, was neutralized with dilute sodium hydrate just previous to making the determination. In an experiment, where the peroxide was not neutralized, it required 44 minutes for 5 gms. of soil to evolve 100 c. c. of oxygen, from 20 c. c.

of peroxide, but in a similar determination, where the peroxide was neutralized, only 9.8 minutes were required. Catalase is similar to other enzyms in being thus sensitive to the reaction of the medium. O. Loew has shown that the activity of *Beta* catalase is destroyed by 1% sulfuric acid, but that its activity is increased by weakly alkaline solutions. Hence the importance of keeping the medium absolutely neutral.

(5) The temperature at which the reaction takes place affects somewhat the speed with which the peroxide is decomposed. A difference of a few degrees is sufficient to seriously affect the agreement of duplicate determinations. The results in Table IX were all obtained with 5 gms. of the same soil sample, and the time given for any single temperature represents the average of a number of determinations at that temperature.

TABLE IX

Temperature in degrees centigrade	Minutes required to evolve 100 c.c. O_2
29	10.9
30.5	10.3
31	10.2
31.5	9.7
32.5	8.5
33	8.1

(6) The manipulation of the experiment has as much effect on the speed of reaction as the acidity of the solution or the concentration of the peroxide. Shaking the flask which contains the soil and peroxide has a tendency to greatly increase the speed at which the oxygen is given off. When the flask was not shaken at all 5 gms. of soil required 57 minutes to produce 100 c. c. of oxygen from 20 c. c. of peroxide; when the flask was shaken once per minute the time required was 15.7 minutes; shaken twice per minute the time was 11 minutes; and shaken continually the time was 10 minutes.

The effect of mechanical motion upon the reaction with peroxide was noticed by Loew but he gave no reason for it. The acceleration of the reaction produced by shaking appears to be due, partly to the greater ease with which the oxygen may escape from the solution, but principally to the fact that when the flask is shaken continually the peroxide is always kept uniformly distributed throughout the solution. If the flask is not shaken a layer of peroxide of low concentration is formed in contact with the soil and this layer is only slowly displaced by peroxide of a higher concentration through diffusion.

From a consideration of the preceding it is evident, that if the catalase content of soils is to be compared with any degree of accuracy, there are several conditions to be fixed. With this in view a constant method was adopted.

Five gms. of soil, prepared as for analysis, were placed in a 300 c. c. Erlenmeyer flask and allowed to stand over night with 40 c. c. of water. Just previous to making the determination of the oxygen evolved, 20 c. c. of 2.75% peroxide were neutralized with dilute caustic soda using phenophthalein as an indicator. The 20 c. c. of neutralized peroxide were run into the flask through a dropping funnel and the oxygen evolved was caught in an inverted burette. During the course of the reaction the flask containing the soil and peroxide was shaken continually and the volume of oxygen was recorded every minute. The temperature at which the reaction took place was measured by a thermometer inserted through the stopper with the bulb in the solution. As the reaction proceeded a slight amount of heat was given off, which did not raise the temperature of the solution more than one degree, unless the reaction happened to be very rapid.

In brief, the catalase contents of soils were compared on the basis of the number of minutes required by 5 gms. of soil to give off 100 c. c. of oxygen from 60 c. c. of neutral .92% peroxide, the flask being shaken continually during the reaction.

The time the soil stood in contact with water previous to making the determinations was found to have some slight effect. So in the method adopted all the soils were allowed to stand some 18 hrs. in contact with water in order that there might not be any variation due to this factor. No attempt was made in any of the work to distinguish between *Alpha* and *Beta*-catalase. And no corrections were made in the volumes of oxygen for variations in temperature and barometric pressures as the accuracy of the method did not warrant it.

The accuracy of the method was tested by making some fifty determinations with the same soil at different temperatures. It was found that while duplicate or triplicate determinations with the same soil made on the same day repeatedly agreed within two or three tenths of a minute, duplicate determinations of the same soil made on different days often varied from each other by as much as a minute. This variation was probably mostly due to a difference in the rate at which the flask was agitated, as it is impossible to always shake the flask by hand at a uniform rate. Six determinations of the same soil made on different days at 30.5° C gave an average of 10.3 minutes, the maximum time being 11 min. and the minimum 9.7 min. Seven determinations at 32° averaged 8.8 min., with a maximum of 9.1 min. and a minimum of 8.6. Four determinations at 31° averaged 10.2 min., with a maximum of 10.5 min. and a minimum of 9.7 min. It is probable that with a mechanical shaker very constant results could be obtained.

EFFECT OF DIFFERENT SOIL TREATMENTS UPON THE CATALASE CONTENT.

HEAT.

It was found the catalase in soils was much more resistant to heat than preparations of the enzym. Loew found that crude

preparations of *Alpha* and *Beta* catalase were killed by heating for one minute at 80° C. But if the catalase is not separated from what it is associated with in the soil it is little affected by heating at 100° C. It will be seen from Table X that heating at 100° C for one half hour destroyed only two tenths of the active amount of catalase, and that heating for seven hours diminished the activity less than half.

TABLE X

Temperature at which 0_2 evolved	Mins. required to evolve 100 c. c. 0_2	Average No. of mins. required for same temp. by unheated soil.	Proportion of catalase undestroyed by heating	Hours soil heated at 100°
29°	12.7	10.5	.8	½
30.5	13.6	9.5	.7	1
32	12.8	8.8	.7	2
32	13	8.8	.7	3
32	13.5	8.8	.7	4
33	13.1	8.4	.7	5
34	11.8	7.3	.6	6
34	13	7.3	.6	7

But heating at 100° C for thirty minutes reduced the activity to .6 of the normal, and heating at 121° for fifteen minutes reduced the activity to .7 of normal. It was found that exposure to a high temperature for a short time had less effect than the action of a lower temperature for a longer time. Thus heating at 130° for five minutes only reduced the activity to .8 of normal.

ACTION OF CARBON BISULPHIDE.

A small amount of carbon bisulphide added to the water in which the soil stood had the effect of depressing considerably the activity of the catalase. One tenth of a c. c. of CS_2 added to 5 gms. of soil depressed the activity to .6 of the normal and .5 of a c. c. lowered the activity to .4 of normal.

EFFECT OF MANURES.

Treatment of the soil with the ordinary manures did not appear to affect the activity or content of catalase. A large sample of soil was prepared and put in pots, four pounds to the pot. The manures were applied and the soil was kept moist and occasionally stirred. At the end of three months each pot was sampled and a determination made of the catalase content, which was compared with that of soil similarly exposed but untreated with manures. The following fertilizers showed no effect.

Amonium sulphate	at	the	rate	of	200,	500	&	1000	lbs.	per acre
Sodium nitrate	"	"	"	"	"	"	"	"	"	"
Cyanamid	"	"	"	"	"	"	"	"	"	"
Lime	"	"	"	"	4000	lbs.			"	"
Cowpeas	"	"	"	"	5	tons			"	"
Goat manure	"	"	"	"	4	"			"	"
Tankage	"	"	"	"	400	lbs.			"	"
Dried Blood	"	"	"	"	400	"			"	"
Bone Meal	"	"	"	"	400	"			"	"

Potassium sulphate 400 lbs. + sodium nitrate 400 lbs. + Acid phosphate 400 lbs. per acre.

Potassium sulphate 400 lbs. + sodium nitrate 400 lbs. + acid phosphate 400 lbs. + 2000 lbs. lime.

The addition of 25 c.c. of dialyzed ferric hydrate per pot was also without effect.

CONCLUSIONS.

The power of a soil for decomposing hydrogen peroxide depends upon the catalase content, enhanced in special cases only by the colloidal action of the mineral constituents.

This property is not a measure simply of the bacterial content, but is a rough measure of the combined quantity of bacteria and organic matter present in the soil.

The most accurate method of comparing different quantities of catalase is on the basis of the time required to evolve a certain volume of oxygen from a certain quantity and concentration of peroxide.

The speed of the reaction between catalase and hydrogen peroxide is dependant upon:

1 The amount of catalase present,
2 The concentration of the peroxide used,
3 The amount of peroxide used,
4 The acidity-or alkalinity of the solution in which the reation takes place,
5 The temperature at which the reaction takes place,
6 The frequency with which the flask containing the catalase and peroxide is agitated.

In the method adopted the catalase contents were compared on the basis of the number of minutes required by 5 gms. of soil to evolve 100 c. c. of oxygen from 60 c. c. of neutral .92% peroxide, the flask being shaken continually during the reaction.

Exposure to a high temperature for a short time had less effect in destroying catalase in soils than the action of a lower temperature for a longer time.

Carbon bisulphide inhibits materially the catalytic action.

Treatment of soil with manures was without effect on the activity or amount of catalase.